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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification<sup>5</sup> : A61K 31/675, 31/495, 31/47 A61K 45/06 // (A61K 31/675 A61K 31:19) (A61K 31/495 A61K 31:19) (A61K 31/47 A61K 31:19)</p>	<p>A1</p>	<p>(11) International Publication Number: <b>WO 93/04</b></p> <p>(43) International Publication Date: 18 March 1993 (18.03)</p>
<p>(21) International Application Number: PCT/US92/07540</p> <p>(22) International Filing Date: 2 September 1992 (02.09.92)</p> <p>(30) Priority data: 756,401 9 September 1991 (09.09.91) US</p> <p>(71) Applicant: WARNER-LAMBERT COMPANY [US/US]; 2800 Plymouth Road, Ann Arbor, MI 48105 (US).</p> <p>(72) Inventors: BIGGE, Christopher, Franklin ; 1811 Packard, Ann Arbor, MI 48104 (US). JOHNSON, Graham ; 11 Pine Drive, Madison, CT 06443 (US). TAYLOR, Charles, Price, Jr. ; 405 Madison Street, Chelsea, MI 48118 (US). WELTY, Devin, Franklin ; 2213 Placid Way, Ann Arbor, MI 48105 (US).</p>		<p>(74) Agents: DAIGNAULT, Ronald, A.; Warner-Lam Company, 2800 Plymouth Road, Ann Arbor, MI 48 (US) et al.</p> <p>(81) Designated States: AU, CA, CS, FI, HU, JP, KR, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GR, IE, IT, LU, MC, NL, SE).</p> <p>Published With international search report.</p>
<p>(54) Title: PHARMACEUTICAL PREPARATION CONTAINING AN URICOSURIC AND AN EXCITATORY AMINO ACID ANTAGONIST</p> <p>(57) Abstract</p> <p>The present invention is a pharmaceutical composition having a combination of an uricosuric agent such as probenecid the like and an excitatory amino acid antagonist useful for treating a disease advantageously affected by treatment with an ex citatory amino acid antagonist.</p>		

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PHARMACEUTICAL PREPARATION CONTAINING AN URICOSURIC AND AN  
EXCITATORY AMINO ACID ANTAGONIST

5

BACKGROUND OF THE INVENTION

10 The present invention is a pharmaceutical  
composition and method for coadministration of a  
uricosuric agent and the like, such as probenecid or  
sulfipyrazone and an excitatory amino acid (EAA)  
antagonist, including, for example, an AMPA  
antagonist, a strychnine insensitive glycine  
15 antagonist, or a competitive NMDA antagonist. This  
invention results in a significant increase in the  
duration of action and magnitude of effect for such  
EAA antagonists.

20 Numerous excitatory amino acid antagonists have  
been described which potently inhibit the activation  
of both N-methyl-D-aspartate (NMDA) and AMPA and  
kainic acid receptor associated ion channels in  
vitro. However, in vivo activity in a range of  
pharmacological models has often been shown to be  
25 significantly less than expected from a knowledge of  
the in vitro receptor binding profile. This  
diminished in vivo activity is thought to result from  
limited access of these compounds to the brain  
resulting from an inability to pass passively through  
the blood brain barrier (BBB). Poor blood brain  
30 barrier permeability has often been observed or  
inferred for polar or hydrophilic compounds (see, for  
example, R. P. Compton, et al, in Neuroscience  
Letters 84:339-344 (1988) and A. G. Chapman, et al,

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in Neuroscience Letters 37:75-80 (1983). However, in addition to an inability to cross the blood brain barrier, the inability to establish a sufficient concentration of a pharmacological agent in the brain may also result from the existence of unidirectional transport systems which are known to exist in the luminal wall of the endothelial cells which comprise the blood brain barrier. See R. Spector, in Pharmacology 40:1-7(1990). The suggested endogenous function of such transport systems is to remove unwanted biological molecules and metabolic byproducts from the cerebrospinal fluid surrounding the brain. However, in the case of a desirable pharmacological agent, such transport systems may instead act to limit inappropriately the access of such compounds into the brain. The result of this situation is that to achieve sufficient brain concentrations of such pharmacological agents necessary for the desired pharmacological effect to be observed, compounds are required to be given in higher doses and at more frequent intervals. This increased brain penetration is often achieved through elevating serum drug concentrations to the point where significant peripheral side effects or organ toxicity are evident. Compounds which would act to inhibit the operation of such unidirectional brain endothelial transport systems would therefore be predicted to reduce the amount of a pharmacological agent required to elicit a pharmacological effect in addition to prolonging its duration of action. Such a reduction in required dosing would, of course, minimize the occurrence of peripheral side effects and limit specific organ toxicity.

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5 Much is known about the effects; such as on  
renal excretion, of uricosuric drugs (see Gutman,  
"Uricosuric Drugs, with Special Reference to  
Probenecid and Sulfipyrazone", Advances in  
10 Pharmacology 4:91-142 (1966)) and particularly  
probenecid (see Beyer, "Factors Basic to the  
Development of Useful Inhibitors of Renal Transport  
Mechanisms," Arch. Int. Pharmacodyn. XCVII(1):97-117  
(1954); Weiner, et al, "On the Mechanism of Action of  
15 Probenecid on Renal Tubular Secretion," Bull. Johns  
Hopkins Hospital 156:333-346 (1960); Hedaya, et al,  
"Probenecid Inhibits the Metabolic and Renal  
Clearances of Zidovudine (AZT) in Human Volunteers",  
Pharmac. Res. 7(4):411-417 (1990), and "Probenecid",  
20 Goodman and Gilman, 8th ed. pp 745-746).

15 In fact, generally, Cunningham, et al, in  
"Clinical Pharmacokinetics of Probenecid", Clinical  
Pharmacokinetics 6:135-151 (1981) stated "Most of the  
drug-drug interactions involving probenecid are due  
20 to an effect on the kidney block of transport of  
acidic drugs."

As early as 1950 another effect of probenecid  
was recognized on paraaminosalicylic acid and  
penicillin (see Boger, et al, "The Influence of a New  
25 Benzoic Acid Derivative of the Metabolism of  
Paraaminosalicylic Acid (PAS) and Penicillin" as  
presented before the 31st Annual Session of the  
American College of Physicians, Boston, April, 1950).  
Other disclosures of the effects of probenecid are  
30 found as follows:

Gibaldi, et al, "Apparent Effect of Probenecid  
on the Distribution of Penicillins in Man", Clinical  
Pharmacology and Therapeutics 9(3):345-349;  
Dewhurst, K., "The Use of Probenecid for Increasing

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Penicillin Concentrations in Cerebro-Spinal Fluid",  
Acta Neurol. Scandinav. 45:253-256 (1969);  
Sjöström, R., "Steady-State Levels of Probenecid and  
Their Relation to Acid Monoamine Metabolites in Human  
Cerebrospinal Fluid", Psychopharmacologia (Berl.)  
25:96-100 (1972).

Spector, R. and Lorenzo, A. V., "The Effects of  
Salicylate and Probenecid on the Cerebrospinal Fluid  
Transport of Penicillin, Aminosalicylic Acid and  
Iodide", The Journal of Pharmacology and Experimental  
Therapeutics 1988(1):55-65 (1974); Roos, et al,  
"Quantitation of CSF Concentrations and Biological  
Activity of Probenecid Metabolites", Eur. J. Clin.  
Pharmacol. 17:223-226 (1980); Van Der Poel, F. W., et  
al, "Evidence for a Probenecid-Sensitive Transport  
System of Acid Monoamine Metabolites from the Spinal  
Subarachnoid Space", Psychopharmacology 52:35-40  
(1977); Bode, et al, "Active Transport of  
Methotrexate from Cerebrospinal Fluid in Humans",  
Cancer Research 40:2184-2187 (July 1980);  
Hedaya, M. A. and Sawchuk, R. J., "Effect of  
Probenecid on the Renal and Nonrenal Clearances of  
Zidovudine and its Distribution into Cerebrospinal  
Fluid in the Rabbit, J. of Pharmaceutical Sciences  
78(9):716-22 (September 1989); Sawchuk, R. J. and  
Hedaya, M. A., "Modeling the Enhanced Uptake of  
Zidovudine (AZT) into Cerebrospinal Fluid. I. Effect  
of Probenecid, Pharmaceutical Research 7(4):332-338  
(1990).

Studies regarding excitatory amino acid  
antagonist pharmacokinetics provide no basis to lead  
an ordinary artisan to the differences of the present  
invention. See Chapman, et al, "Uptake of a Novel  
Anticonvulsant Compound, 2-Amino-7-Phosphono-



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[4,5-<sup>3</sup>H]Heptanoic Acid, into Mouse Brain",  
Neuroscience Letters 37:75-80 (1983) and Compton, et  
al, "Determination of the Pharmacokinetics of  
2-Amino-7-Phosphonoheptanoate in Rat Plasma and  
5 Cerebrospinal Fluid", Neuroscience Letters 84:339-344  
(1988).

Specifically, I. McDonald of Merrell-Dow  
presented a talk at the Excitatory Amino Acid  
Symposium held at the American Chemical Society  
10 Spring National Meeting held in Atlanta, Georgia on  
April 15, 1991 in which he stated that preliminary  
metabolic studies show longer duration of protection  
against seizures induced by quinolinic acid in mice  
when pretreated with probenecid followed by the  
15 administration of a compound of the formula  
4-[(carboxymethyl)imino]-5,7-dichloro-  
1,4-dihydro-2-quinolinecarboxylic acid (MDL 100,748),  
This was shown by the following data:

20 Preliminary Metabolic Studies

4-[(Carboxymethyl)imino]-5,7-dichloro-  
1,4-dihydro-2-quinolinecarboxylic Acid (MDL 100,748)

25 Effect of MDL 100,748 (65 mg/kg; IV) in the  
quinolinic acid seizure model in mice.

	<u>Time of Administration of</u> <u>MDL 100,748 Prior to QA</u>	<u>Protection</u>
30	5 min	9/10
	30 min	0/10

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Mice pretreated (30 min) with probenecid 200 mg/kg (IP) 30 minutes prior to receiving 65 mg/kg (IV) of MDL 100,748.

5

<u>Time of Administration of MDL 100,748 Prior to QA</u>	<u>Protection</u>
5 min	9/10
30 min	7/10

10

However, the prolonged protection provided by probenecid described in the talk was attributed to the reduced metabolism resulting from inhibition of renal transport mechanisms. This differs from the present invention now found to provide reduced amounts of antagonist for effects equivalent to previously known larger amounts.

15

The present invention differs from the oral disclosure as it is based on prevention of the unilateral transport of EAA acid competitive NMDA antagonists from brain to blood by coadministration of probenecid and related compounds. In other words, the method of the present invention prolongs the effect of these antagonists by interfering with the efflux of the antagonists from the site of their activity, not inhibition of renal excretion.

20

25

The coadministration of the present invention is different from that expected by an ordinarily skilled artisan from the above-noted disclosures. It is now found that the predominant pharmacokinetic effect of probenecid on the EAA and competitive NMDA antagonists in the present method of coadministration is based on the previously unappreciated

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unidirectional effect on the antagonists in relation to the blood brain barrier. Generally, Kang, et al, "Acidic Drug Transport In Vivo Through the Blood-Brain Barrier. A Role of the Transport Carrier for Monocarboxylic Acids", in J. Pharmacobio-Dyn., 13:158-163 (1990) teach "acidic drugs could be transported by a carrier-mediated system for monocarboxylic acids at the BBB (blood brain barrier) and the transport system was not changed by the disease state."

Further, Oldendorf, W. in "Certain Aspects of Drug Distribution to Brain", (1976) provides a discussion of differing elements of blood brain barrier permeability describing a flow-limited distribution. It is now found the present method of coadministration provides a positive effect on the availability of EAA antagonists in the brain contrary to the flow-limited distribution for antagonist administration alone.

Certainly no predictability regarding the results of coadministration of the present invention method is previously shown.

#### SUMMARY OF THE INVENTION

The present invention is a method of treating a disease advantageously affected by an EAA antagonist in a human suffering therefrom comprising coadministration of a uricosuric agent, preferably probenecid, and (1) an EAA antagonist. The amount of agent and the amount of antagonist is an optimally effective amount of each when administered together for the treatment of the disease.

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5 The present invention is a pharmaceutical composition for treating a disease advantageously affected by an excitatory amino acid antagonist which comprises an amount of probenecid effective to inhibit unidirectional efflux of an amount of the excitatory amino acid antagonist from the brain effective for the treatment of the disease the amount of the excitatory amino acid together with a pharmaceutically acceptable carrier.

10 The present invention is a method for treating a disease advantageously affected by an excitatory amino acid antagonist in a human suffering therefrom comprising the above pharmaceutical composition in unit dosage form.

15 The present invention is also a method for the manufacture of a pharmaceutical composition for treating a disease advantageously affected by an excitatory amino acid antagonist which is an amount of probenecid effective to inhibit efflux from the brain of the antagonist consisting of an excitatory amino acid antagonist and the effective amount of the antagonist to treat the neurodegenerative disease together with a pharmaceutically acceptable carrier.

20 The present invention is a composition and a method for treatment of neurodegenerative disorders.

25 Excessive excitation by neurotransmitters can cause the degeneration and death of neurons. It is believed that this degeneration is in part mediated by the excitotoxic actions of the excitatory amino acids (EAA), particularly of glutamate and aspartate at N-methyl-D-aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-isoxazole propionic acid (AMPA), and kainate receptors. This excitotoxic action is responsible for the loss of neurons in cerebrovascular disorders

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such as cerebral ischemia or cerebral infarction resulting from a range of conditions such as thromboembolic or hemorrhagic stroke, cerebral vasospasm, hypoglycemia, cardiac arrest, status epilepticus, perinatal asphyxia, anoxia such as from drowning, pulmonary surgery and cerebral trauma as well as lathyrism, Alzheimer's, and Huntington's diseases. These compounds may also be useful for the treatment of schizophrenia, epilepsy, anxiety, pain, drug addiction, and emesis.

Suggested therapies for these neurodegenerative diseases include compounds which act specifically as antagonists of EAA receptors and in particular the NMDA receptor (Schwarz, R. and Meldrum, B., The Lancet 140 (1985); Meldrum, B. in "Neurotoxins and Their Pharmacological Implications", edited by P. Jenner, Raven Pres, New York (1987); Choi, D. W., Neuron 1:623 (1988).

The disease advantageously affected by excitatory amino acid antagonists of the present composition and method of use generally includes a disorder responsive to an excitatory amino acid antagonist including, for example, disorders characterized as neurodegenerative. Such diseases or disorders include what are commonly known as stroke, convulsions, migraines or senility but more particularly result from cerebral ischemia; cerebral infarction; cerebral vasospasm; hypoglycemia; cardiac arrest; status epilepticus or cerebral trauma. Conditions also responsive to excitatory amino acid antagonists include; schizophrenia; epilepsy; pain; anxiety; neurodegenerative disorders such as Alzheimer's disease; Huntington's disease; or control of emesis. Diseases preferably treated with an

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excitatory amino acid antagonist are: convulsions or epilepsy or stroke.

#### BRIEF DESCRIPTION OF THE DRAWINGS

5

The drawings consist of Figures 1, 2, 3, and 4.

FIG. 1 shows the effect of anticonvulsant action over time of 25 mg/kg of 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline, NBQX; administered by intravenous injection, identified hereinafter, in 10 mice as ● and the effect of anticonvulsant action over time of 25 mg/kg of NBQX also administered by intravenous injection in mice pretreated (30 minutes before injection of NBQX) by intraperitoneal injection of 200 mg/kg of probenecid V.

FIG. 2 shows the prevention of tonic extensor seizures from maximal electroshock in mice by NBQX administration with (●) and without (V) combined treatment with probenecid, 200 mg/kg intraperitoneally. The smooth curves are the best fit to the data by probit analysis (J. T. Litchfield and F. Wilcoxon in Journal of Pharmacology 96:99-113, 1949). ED<sub>50</sub> values from probit analysis are shown, along with 95% confidence intervals (in parentheses).

FIG. 3 shows the effect of anticonvulsant action over time of 3 mg/kg, of 4-(3-phosphonopropyl)-2-piperazinecarboxylic acid, CPP administered by intravenous injection in 10 mice as ● and the effect of anticonvulsant action over time of 3 mg/kg of CPP administered by intravenous injection in mice pretreated (30 minutes before injection of CPP) by intraperitoneal injection of 200 mg/kg of probenecid V.

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FIG. 4 shows the percent of the brain protected by 5,7-dichlorokynurenic acid (co-injected) on NMDA-induced brain injury in rat without pretreatment by probenecid shown as a nanomolar dose by ● and with ip pretreatment with 200 mg/kg of probenecid at 30 minutes before injection shown as ∇.

## DETAILED DESCRIPTION

The excitatory amino acid antagonist of the composition and method of the present invention include each of the compounds of the following patents or applications which are incorporated by reference therefor:

- I. Quinoxaline diones in  
EP 377,112-A; EP 374534-A; EP 348872-A;  
EP 315959-A; EP 260467-A; US 4,889,855; and US  
Patent No. 4812458;
- II. Quinoxalinic acid derivatives in US application  
numbers 624,156 filed December 7, 1990; and  
631,139 filed December 20, 1990;
- III. Indole derivatives in the following patents and  
copending applications:  
US 4960786, EP 396124, and US application  
numbers 670,860 filed March 18, 1991; 699,875  
filed May 14, 1991, and 705,022 filed May 24,  
1991;
- IV. Kynurenic acid derivatives:  
EP 386839-A and EP 303387;
- V. Pyrrolidinone derivatives:

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US 4925867, US 4863953, and GB 2231048-A;

VI. D-cycloserine, its prodrugs and admixtures with other compounds in:

5 EP 387867, EP 378134, and US 4904681;

VII. Isatine derivatives in:

10 US application numbers 624,157 filed December 7, 1990; 624409 filed December 7, 1990, and 670,061 filed March 15, 1991; also Ref. No. NS12.

15 The competitive NMDA antagonist of the composition and method of the present invention include each of the compounds of the following patents or applications which are incorporated by reference therefor:

20 GB2104078-A; EP 420806-A, EP 391850, US Patent No. 4906621, US Patent No. 4898854, EP 302826-A, US Patent No. 4746653, EP 275820, US Patent No. 4705781, US Patent No. 4968678, US Patent No. 4902687, US Application No. 902695, EP 418863-A; US Patent No. 4761405, US Patent No. 4657,899; Parke-Davis: Applications as follows: GB 2157685-B; GB 2198134-B, 25 GB 2201676-A, GB 2156818-B; US Patent No. 4918064, EP 364996-A, EP 342558.

30 Effects of each of the above-noted antagonists (EAA, including, for example, an AMPA antagonist, a strychnine insensitive glycine antagonist, or competitive NMDA antagonist) are centrally mediated by the uricosuric agent, preferably probenecid in the method of the present invention so that the effects



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of each of the compounds is extended and its effectiveness increased per dose of antagonist without increased side effects. These effects are due to higher amounts of the antagonists of the present invention in the brain because probenecid inhibits unidirectional transport from the brain. Clearly this differs from inhibition of renal excretion as set out in the oral disclosure above since such inhibition of excretion would be understood to increase blood levels of the antagonists throughout the body. Under this rationale, the antagonist-induced peripheral side effects would be expected to increase when combined with probenecid rather than as in the present invention provide for a lower dose of probenecid for greater effectiveness at the same time minimizing peripheral side effects.

Preferred combinations of the present invention include NBQX and probenecid; and CPP and probenecid.

According to this invention a combination of either an EAA antagonist or a competitive NMDA antagonist and preferably probenecid is administered in an amount effective to treat a neurodegenerative disease.

The combination comprises a daily dose of an uricosuric agent of about 1 g/day to 4 g/day and preferably about 2 g/day of probenecid. This may be given in four divided portions of 500 mg/dose (see Goodman and Gilman, "Probenecid", 8th Ed., pages 745-746; Selen, et al, "Pharmacokinetics of Probenecid Following Oral Doses of Human Volunteers", J. of Pharma. Sci. 71(11) (November 1982), and Selen, A., "Dose Dependent Pharmacokinetics of Probenecid Following Single and Repeated Doses", a

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thesis for the degree of Doctor of Philosophy from the University of Wisconsin-Madison.

For the EAA antagonists of the present invention the combination also comprises an amount of antagonist which is approximately tenfold less than for antagonist alone for comparable activity in the treatment of the neurodegenerative disease. For example, for NBQX alone (defined hereinafter), the dosage range is from 0.1 mg/kg to 1 mg/kg with a daily dose from 0.5 mg/kg/day to 5.0 mg/kg/day totally but for the present invention the dosage is from 0.01 mg/kg up to less than 0.1 mg/kg, with a daily dose from 0.02 mg/kg/day to 0.2 mg/kg/day such that NBQX need only be given twice daily rather than 4 to 5 times daily.

For a competitive NMDA antagonist of the present invention the combination also comprises an amount of antagonist which is approximately twofold less than for antagonist alone. For example, for CPP alone the dosage range is from 0.03 mg/kg to 0.30 mg/kg with a daily dose of from 0.10 mg/kg/day to 1.0 mg/kg/day totally but for the present invention the dosage is from 0.015 mg/kg up to but not including 0.15 mg/kg with a daily dose from 0.05 mg/kg/day to 0.5 mg/kg/day.

The pharmaceutical compositions of the invention can take any of a wide variety of oral and parenteral dosage forms. The dosage forms comprise as the active components an antagonist; either an EAA antagonist or a competitive NMDA antagonist, as defined previously and an uricosuric agent preferably probenecid as defined previously.

For preparing pharmaceutical compositions, one uses inert, pharmaceutically acceptable carriers that

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can be either solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets, and suppositories. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, or tablet disintegrating agents; it can also be an encapsulating material. In powders, the carrier is a finely divided solid which is in admixture with the finely divided active compounds. In the tablet, the active compounds are mixed with carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain from 5% or 10% to about 70% of active ingredients. suitable solid carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methyl cellulose, sodium carboxymethyl cellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is intended to include the formulation of the active compounds with encapsulating materials as carrier, providing a capsule in which the active components (with or without other carriers) are surrounded by carrier, which are thus in association with it. Similarly, cachets are included. Tablets, powders, capsules, and capsules can be used as solid dosage forms suitable for oral administration.

Liquid form preparations include solutions, suspensions, and emulsions. As an example may be mentioned water or water-propylene glycol solutions for parenteral injection. Liquid preparations can also be formulated in solution in aqueous polyethylene glycol solution. Aqueous solutions

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5        suitable for oral use can be prepared by dissolving the active component in water and adding suitable colorants, flavors, stabilizing, and thickening agents as desired. Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active components in water with viscous material, i.e., natural or synthetic gums, resins, methyl cellulose, sodium carboxymethyl cellulose, and other well-known suspending agents.

10        Preferably, the pharmaceutical preparation is in unit dosage form. In such form, the preparation may be subdivided into unit doses containing appropriate quantities of antagonist and probenecid individually or as a combination, i.e., in a mixture. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, for example, packeted tablets, capsules, and powders in vials or ampoules. The unit dosage form can also be a capsule, cachet, or tablet itself or it can be the appropriate number of any of these in packaged form. Additionally, the unit dosage form may be a dividable form having antagonist in one part and probenecid in the other part, such as, a dividable capsule, a dividable package, or a two-part ampoule, vial or the like.

25        The quantity of antagonist and probenecid in a unit dose of preparation may be varied or adjusted from about 0.01 mg/kg to 10.0 mg/kg, preferably 0.03 mg/kg to less than 0.1 mg/kg of antagonist together with about 500 mg to 2000 mg of uricosuric agent, preferably about 500 mg of probenecid, according to the particular application and the potency of the active ingredients.

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The pharmaceutical compositions preferably are constituted so that they can be administered parenterally or orally. Solutions of the active compounds as free bases and free acids or pharmaceutically acceptable salts can be prepared in water suitable mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid,

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thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients, into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of the sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of active ingredients plus an additional desired ingredient from a previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well-known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

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It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suitable as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active materials calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (1) the unique characteristics of the active materials and the particular therapeutic effect to be achieved, and (b) the limitation inherent in the art of compounding such active materials for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredients are compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit parenteral dosage form can, for example, contain the principal active compound, i.e. EAA antagonist, in amounts ranging from about 0.5 to about 100 mg, with from about 0.1 to 50 mg being preferred in combination with about 500 mg of probenecid. The daily parenteral doses for mammalian subjects to be treated ranges from 0.01 mg/kg to 10 mg/kg of the EAA antagonist. The preferred daily dosage range is 0.1 mg/kg to 1.0 mg/kg.

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The antagonists described above may form commonly known, pharmaceutically acceptable salts such as alkali metal and other common basic salts or acid addition salts, etc. References to the base substances are therefore intended to include those common salts known to be substantially equivalent to the parent compound and described in the above referenced applications or patents.

The following examples are meant to illustrate the methods for determining critical elements of the invention and are nonlimiting.

## EXAMPLE 1

5,7-Dichloro-1,4-dihydro-4-oxo-2-quinolinecarboxylic acid

Results of two studies in mice show that 5,7-dichloro-1,4-dihydro-4-oxo-2-quinolinecarboxylic acid, a NMDA-site glycine antagonist, crosses the blood-brain barrier but returns at a faster rate to the blood by unidirectional efflux. Following intravenous administration of tritiated 5,7-dichloro-1,4-dihydro-4-oxo-2-quinolinecarboxylic acid ( $[^3\text{H}]$ -5,7-dichlorokynurenic acid) to mice, very little penetration and/or retention of radiolabeled material is observed in brain tissue over a 4-hour period. To determine if 5,7-dichloro-1,4-dihydro-4-oxo-2-quinolinecarboxylic acid does not cross the blood brain barrier or penetrates but is rapidly cleared from the brain. A follow-up study is performed in which tritiated 5,7-dichloro-1,4-dihydro-4-oxo-2-quinolinecarboxylic acid is injected directly into the cerebral ventricle of mice at two dose levels. By 60 minutes postdose 12% and 83.4% of total radioactivity remains in brain tissue from the



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initial concentration following administration of a 0.3 and 1.3 mg/kg dose, respectively. The ratio of area under the brain radioactivity curve (5-120 minutes) for the 1.3 mg/kg to 0.3 mg/kg dose is 12, approximately threefold greater than anticipated based on linear kinetics. This data shows 5,7-dichloro-1,4-dihydro-4-oxo-2-quinoline-carboxylic acid is actively transported from brain to blood by a saturable process. Coadministration of probenecid with 5,7-dichloro-1,4-dihydro-4-oxo-2-quinolinecarboxylic acid slows the active transport process and increases the amount and duration of 5,7-dichloro-1,4-dihydro-4-oxo-2-quinolinecarboxylic acid in brain following IV administration to mice. brain and the ability of probenecid to block active, unilateral transport out of the brain.

## EXAMPLE 2

2,3-Dihydroxy-6-nitro-7-sulfamoylbenzo(F)quinoxaline  
or NBOX

Adult male CF1 strain mice (18 to 24 g body weight) are obtained from Charles River Laboratories. Mice are allowed free access to food and water prior to testing. Maximal electroshock is performed according to conventional methods (Krall, R. L., et al, Antiepileptic drug development: II. Anticonvulsant drug screening, Epilepsia 19:409-428 (1978). Electrical current (50 mA sinusoid at 60 Hz) is applied by metal corneal electrodes for 0.2 sec. This intensity of electrical current is five times the amount needed to produce tonic extensor seizures in untreated mice. Pretreatment of mice with known anticonvulsant drugs prevents tonic extensor seizures in a dose-related

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manner (Krall, R. L., et al, Antiepileptic drug development: II. Anticonvulsant drug screening, Epilepsia 19:409-428 (1978). A drug is prepared for intravenous administration by dissolution in aqueous medium with 0.9% sodium chloride. Probenecid is finely divided with mortar and pestle and suspended in 0.2% carboxymethylcellulose in water and given by intraperitoneal injection 30 minutes before administration of anticonvulsant drugs. (Probenecid (200 mg/kg) followed by intravenous administration of 0.09% sodium chloride vehicle causes no anticonvulsant actions in any of 10 mice.)

That is, groups of ten mice each are pretreated with probenecid (200 mg/kg IP) or saline vehicle. Thirty minutes later each group is given a known anticonvulsant dose of one of several anticonvulsant drugs. The time course of anticonvulsant activity is determined by maximal electroshock testing in groups of mice at various times after administration of the anticonvulsant. Results are expressed as the percentage of mice that failed to have a tonic extensor seizure of hindlimbs (percent of mice protected).

The time course of anticonvulsant action with the known AMPA-type glutamate antagonist NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline) is shown in FIG. 1. With only NBQX (25 mg/kg given intravenously) given after saline vehicle, significant anticonvulsant actions are present until 3 minutes (180 seconds) after dosing, but only insignificant anticonvulsant action is seen 15 minutes (900 seconds) after dosing. However, with coadministration of NBQX and probenecid (200 mg/kg), significant anticonvulsant effects are seen

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15 minutes (900 seconds), 30 minute (1800 seconds),  
1 hour (3600 seconds), and 2 hours (7200 seconds)  
after dosing but not 4 hours (14400 seconds) after  
dosing. These results indicate a marked potentiation  
5 of anticonvulsant effects of NBQX when it is given in  
combination with probenecid.

Other CNS behavioral effects of NBQX are also  
markedly increased by coadministration with  
probenecid, as evidenced by ptosis, profoundly  
10 decreased locomotor activity, greatly delayed  
righting reflex, and somnolence following the  
combination of probenecid with 25 mg/kg NBQX but not  
with administration of 25 mg/kg NBQX and saline.

### 15 EXAMPLE 3

Using procedures identical to those of  
Example 2, but with administration of several  
different doses of NBQX,  $ED_{50}$  values were determined  
by probit analysis (J. T. Litchfield and F. Wilcoxon  
20 in Journal of Pharmacology 96:99-113, 1949), with  
times of testing chosen at approximately the time of  
maximal anticonvulsant effect (30 seconds for NBQX  
alone and 10 minutes for NBQX with probenecid  
coadministration, from Example 1). Results are shown  
25 in Figure 2 with an  $ED_{50}$  value for NBQX alone of  
13.1 mg/kg and for NBQX coadministered with  
probenecid of 2.57 mg/kg. The 95% confidence  
intervals of  $ED_{50}$  values are shown in the figure in  
parentheses. These values differ significantly  
30 ( $p < 0.05$ ), demonstrating a quantitative increase in  
potency of NBQX of approximately fivefold following  
probenecid coadministration.

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## EXAMPLE 4

4-(3-Phosphonopropyl)-2-piperazinecarboxylic acid;  
hydrochloride

5 In an analogous study with the known NMDA-type competitive glutamate antagonist, CPP, anticonvulsant actions are also increased by coadministration with probenecid, even though to a smaller extent (FIG. 3). The duration of significant anticonvulsant action of 3 mg/kg CPP is increased from 120 minutes (2.0 hours) without probenecid to 240 minutes (4.0 hours) with probenecid. The percentage of mice protected from maximal electroshock seizures is also increased by probenecid coadministration at each time point tested. However, although the extent of the improved effect of probenecid is less with CPP than with NBQX, the effect is still quantitatively greater for the coadministered agents.

## EXAMPLE 5

5,7-Dichlorokynurenate

20 The neuroprotective action of 5,7-dichlorokynurenate is assessed using an animal model with young rats. Seven-day-old albino rat pups of either sex are anesthetized and given N-methyl-D-aspartate (NMDA) by injection with a fine hypodermic needle into the right posterior corpus striatum. Four days later, the degree of brain damage from NMDA injection is assessed by comparing wet weights of the injected cerebral hemispheres. Methods are similar to those of JW McDonald et al. in Experimental Neurology 106:289-296 (1989) except that 15 nmol of NMDA is used rather than 25 nmol as in the McDonald method. The neuroprotective action of 5,7-dichlorokynurenic acid is assessed by injecting

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it directly into the striatum of the brain in combination with NMDA. As shown in FIGURE 4, the neuroprotective action of 5,7-dichlorokynurenic at three different doses is significantly enhanced by pretreatment of rat pups with 200 mg/kg probenecid. Administration of probenecid alone did not alter the extent of brain damage in comparison to untreated animals.

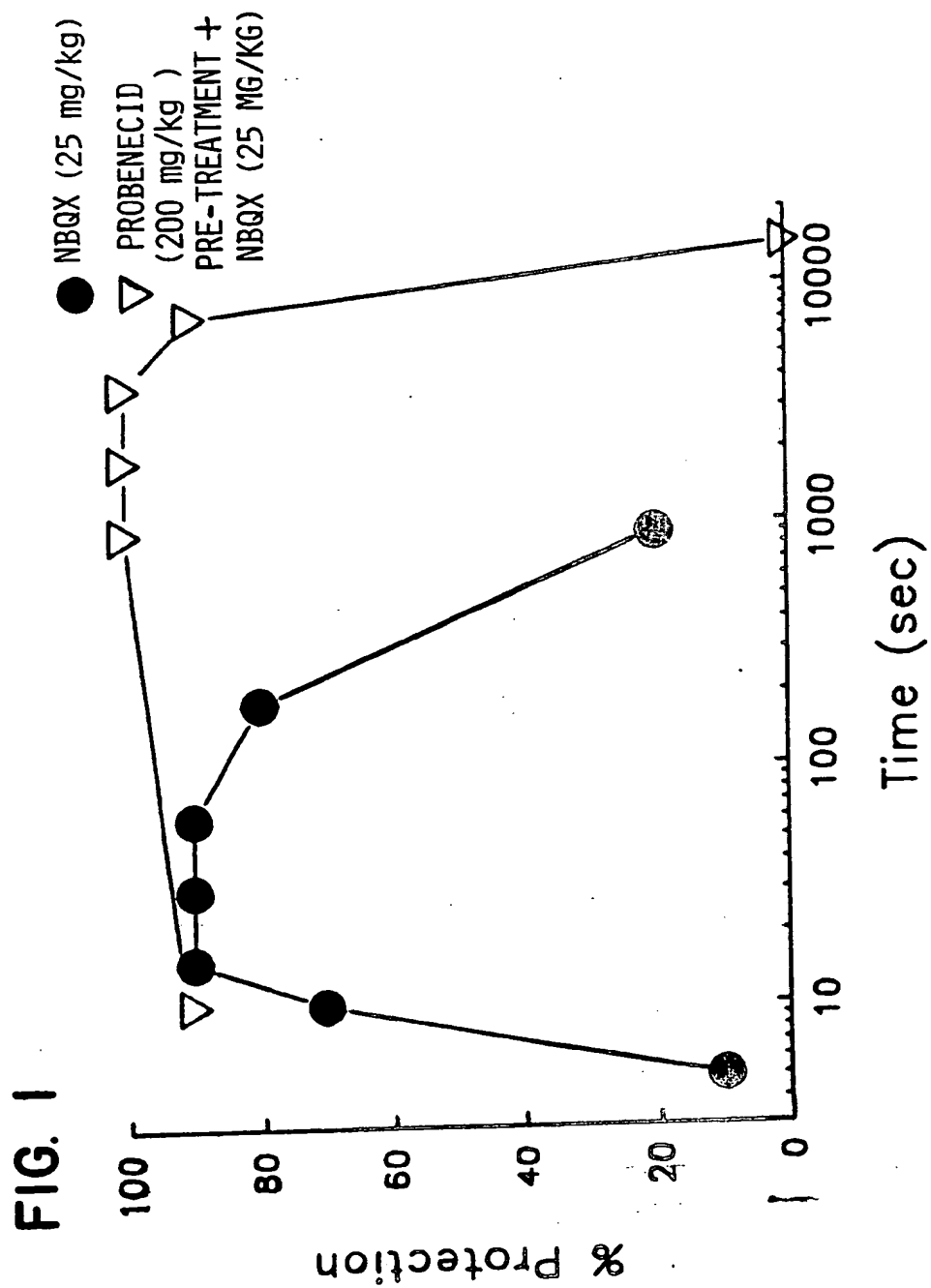
These results support the present invention showing that probenecid pretreatment prevents the transport of 5,7-dichlorokynurenate out of brain tissue and thus increases its neuroprotective action.

## CLAIMS

- 5
1. A pharmaceutical composition for the treatment of a disease advantageously affected by treatment with an excitatory amino acid which comprises an amount of an uricosuric agent effective to inhibit unidirectional efflux from the brain of an inhibited efflux amount of an excitatory amino acid antagonist effective to treat the disease and a pharmaceutical carrier.
  2. A pharmaceutical composition of Claim 1 wherein the uricosuric agent is probenecid.
  3. A pharmaceutical composition of Claim 2 wherein the amount of probenecid is about 500 mg.
  4. A pharmaceutical composition of Claim 3 wherein the antagonist is an excitatory amino acid antagonist.
  5. A pharmaceutical composition of Claim 3 wherein the antagonist is a competitive NMDA antagonist.
  6. A pharmaceutical composition of Claim 3 wherein the antagonist is an AMPA antagonist.
  7. A pharmaceutical composition of Claim 3 wherein the antagonist is a Kainate antagonist.
  8. A pharmaceutical composition of Claim 5 wherein the antagonist is 2,3-dihydro-6-nitro-7-sulfamoyl benzo(F) quinoxaline.

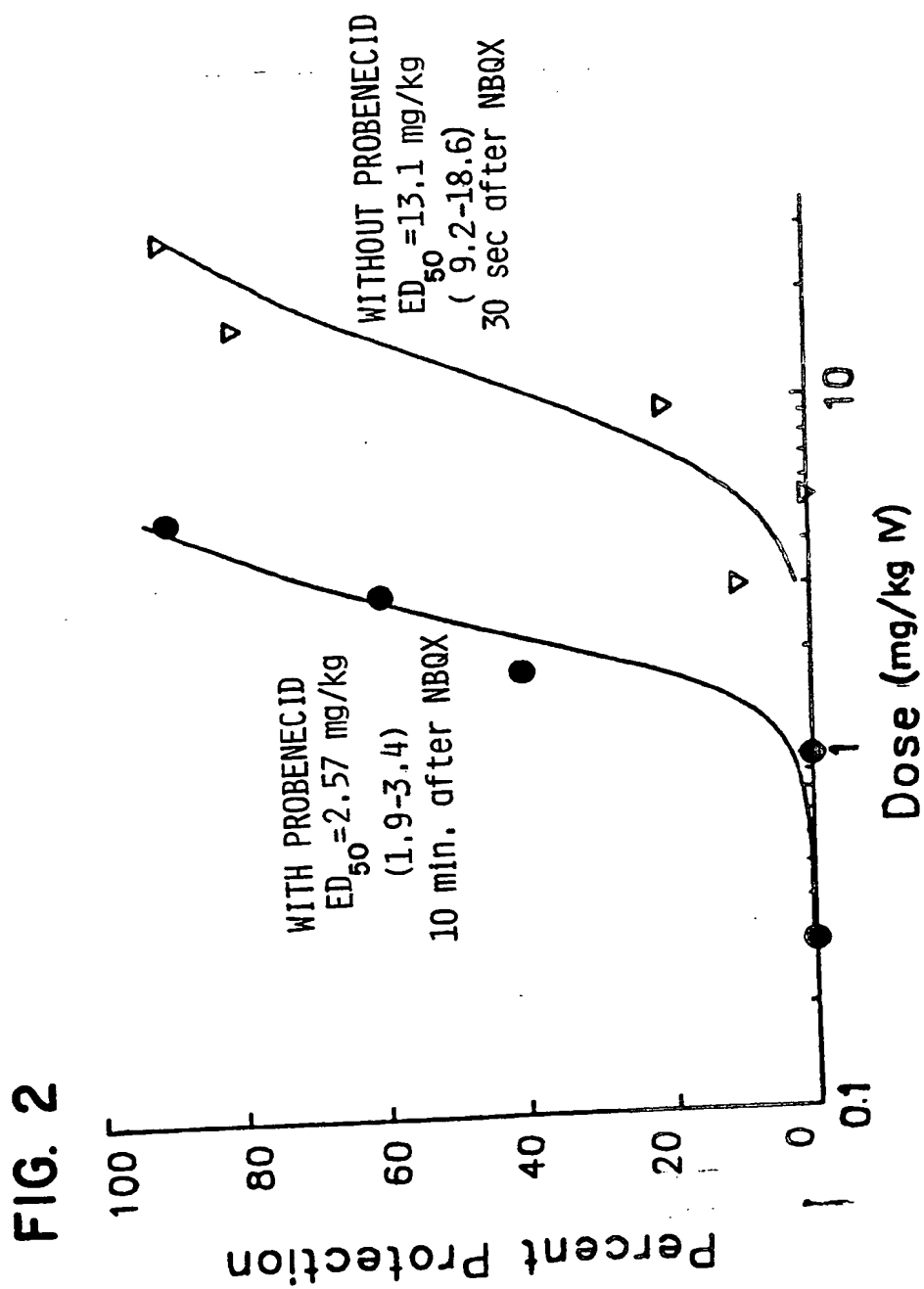
9. A pharmaceutical composition of Claim 5 wherein the antagonist is CPP.
10. A pharmaceutical composition of Claim 5 wherein the antagonist is 5,7-dichlorokynurenic acid.
11. A method of treating a disease advantageously affected by an excitatory amino acid in a human suffering therefrom which comprises the pharmaceutical composition of Claim 1 in unit dosage form.
12. A method of Claim 11 wherein the disease is a neurodegenerative disease.
13. A method of Claim 12 wherein the neurodegenerative disease is stroke.
14. A method of Claim 11 wherein the disease is a neurological disease.
15. A method of Claim 14 wherein the neurological disease is epilepsy, schizophrenia, anxiety, or pain.
16. A method of Claim 14 wherein the neurological disease is epilepsy.

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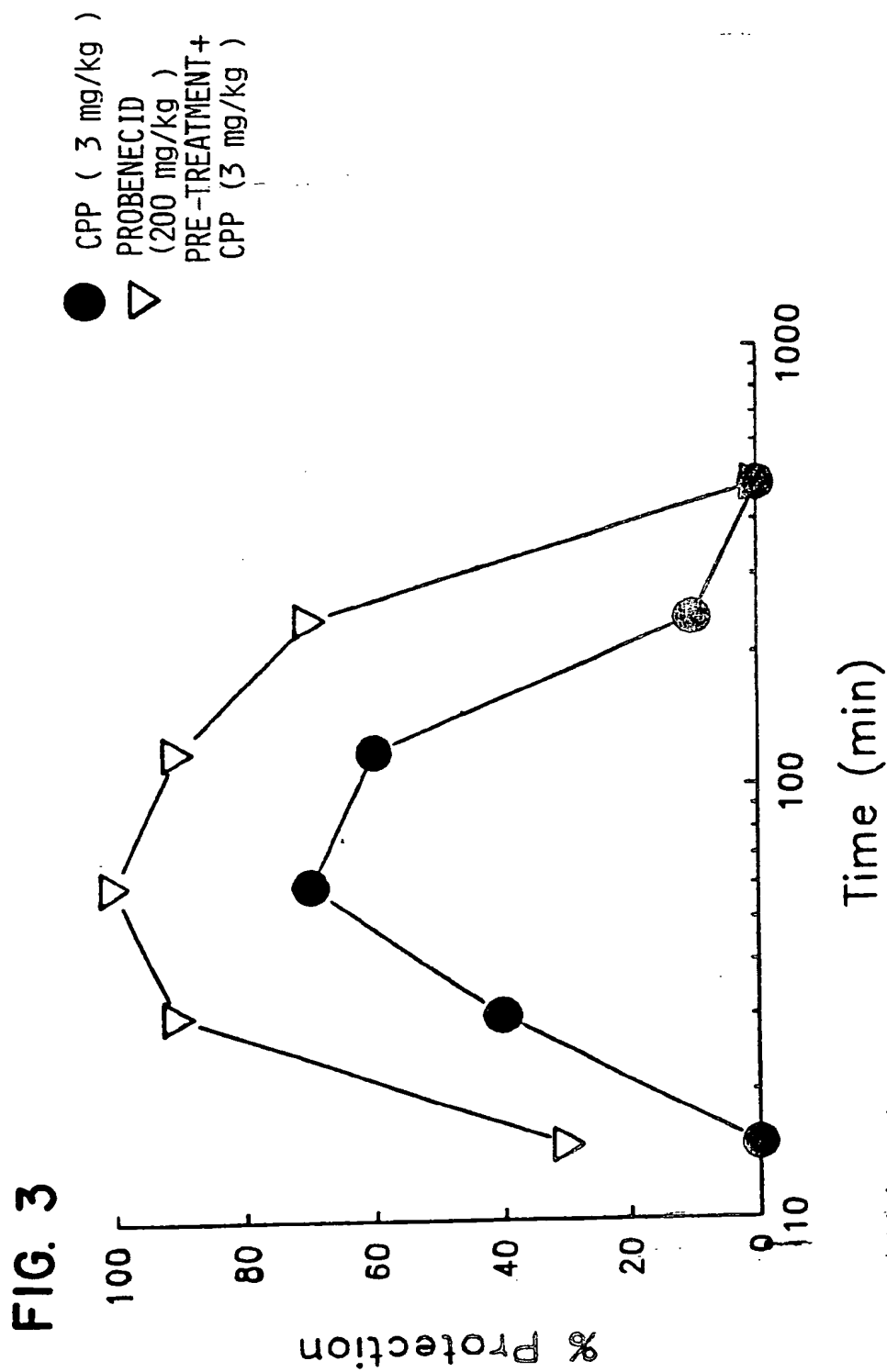




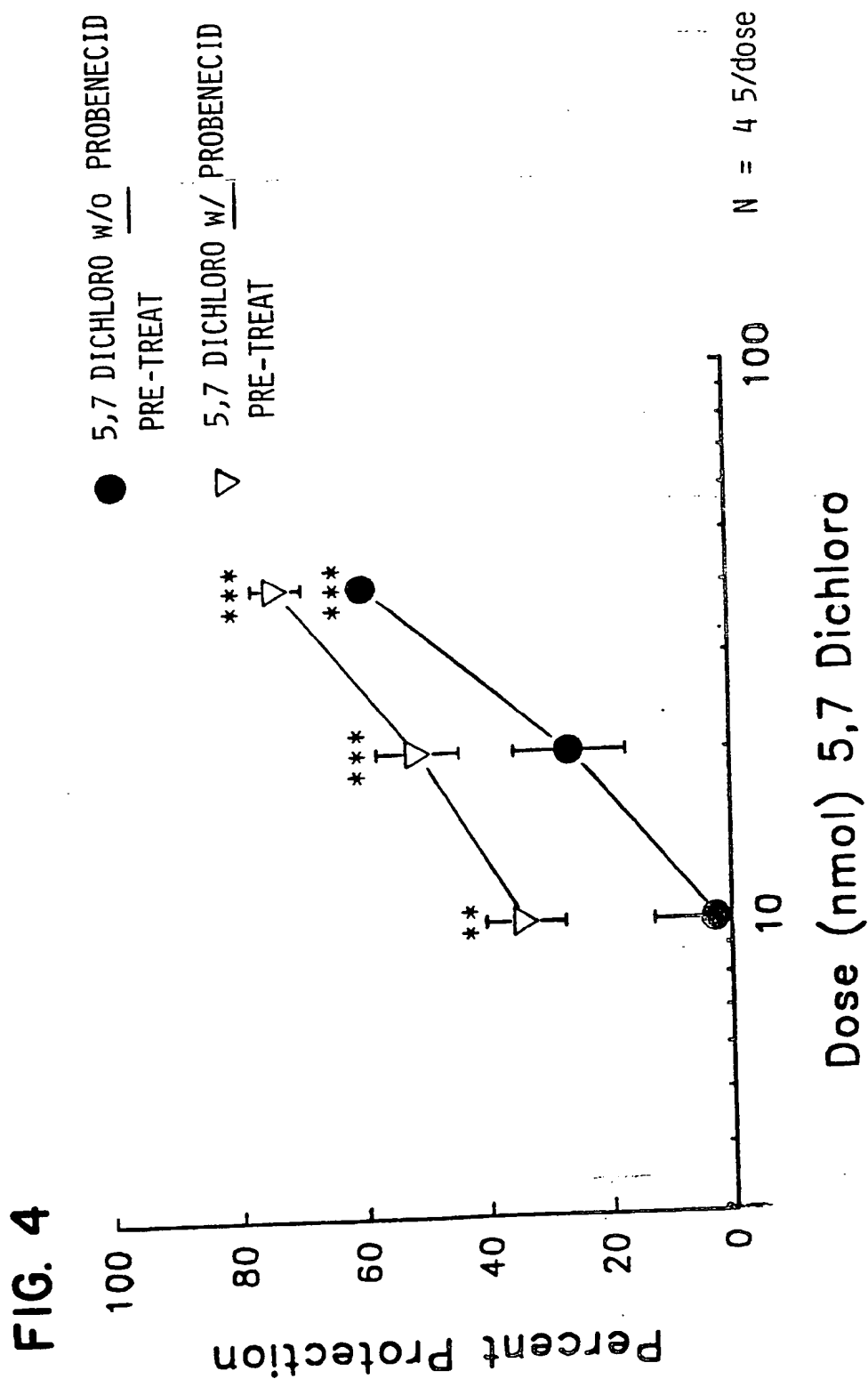
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## INTERNATIONAL SEARCH REPORT

PCT/US 92/075

International Application No

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (If several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 A61K31/675; A61K31/495; A61K31/47; A61K45/06 //A61K31/675,31:19)(A61K31/495,31:19)(A61K31/47,31:19)		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	A61K	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim <sup>13</sup>
E	EP,A,0 501 378 (MERRELL DOW PHARMACEUTICALS INC.) 2 September 1992 see claims	1-16
X,P	EUROPEAN JOURNAL OF PHARMACOLOGY vol. 213, no. 1, 17 March 1992, pages 151 - 153 TAYLOR C.P. & VATANIAN M.G. 'Probenecid pretreatment enhances anticonvulsant action of NBQX in mice' see abstract	1-16
<p><sup>10</sup> Special categories of cited documents : <sup>10</sup></p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
21 DECEMBER 1992		30. 12. 92
International Searching Authority		Signature of Authorized Officer
EUROPEAN PATENT OFFICE		LEHERTE C.F.M.

Form PCT/ISA/210 (second sheet) (January 1983)

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